*ON THE COOPERATIVITY OF BIOLOGICAL MEMBRANES**

By Jean-Pierre Changeux,† Jean Thiéry,‡ Yvonne Tung, and C. Kittel

VIRUS LABORATORY, DEPARTMENT OF CHEMISTRY, AND DEPARTMENT OF PHYSICS, UNIVERSITY OF CALIFORNIA, BERKELEY

Communicated December 5, 1966

The amplifying properties of biological membranes in their responses to ligand binding have already received considerable attention,[1] but the molecular basis of this important behavior is far from being understood. In this communication we wish to apply to this problem some of the principles and approaches recently proposed for regulatory enzymes[2] and to discuss the cooperative properties of membranes on the basis of their highly ordered structure.

Although data on the chemical constitution and structure of biological membranes are still fragmentary and many basic aspects as yet are not clear, the following features have been established and will be considered:

(1) Membranes are made up by the association of repeating globular lipoprotein units.[3]

(2) The conformation of these units differs when they are dispersed in solution or organized into a membrane structure.[4]

(3) A large number of biological or artificial lipoprotein membranes respond *in vivo* as well as *in vitro* to the binding of specific ligands by some modification of their properties which reflects rearrangement of the membrane organization and presumably of the conformation of the repeating units.[5]

(4) In several instances the response curve of a membrane to increased concentrations of ligand deviates from the Langmuir isotherm. Depending on the specific membrane considered, the response curve can be slightly S-shaped ("graded response")[6] or extremely sharp ("all-or-none response").

Theory.—We consider a biological membrane as an ordered collection of repeating globular lipoprotein units, or "protomers," organized into a two-dimensional crystalline lattice:

(1) The protomer constitutes the "primitive cell" of the lattice and does not necessarily possess in itself any particular property of symmetry. (It can be made up of a single polypeptide chain or of several different protein subunits associated with lipids in characteristic amounts.)

(2) Several conformational states are reversibly accessible to the protomer.

(3) The protomer possesses at least one receptor site for each type of specific ligand normally capable of binding to the membrane,[7] and the affinity of one, or several, of these receptor sites toward the corresponding ligand is altered when a transition occurs from one to another conformational state.

(4) The conformation of the protomer depends upon its association with the neighboring protomers in the lattice and thus is submitted to a *lattice constraint* similar to the quaternary constraint involved in the organization of the quaternary structure of oligomeric proteins.[8]

For simplicity we discuss the situation in which *only two*[9] conformational states ($R \rightleftharpoons S$) are accessible to the protomer and in which a single receptor site specific for the considered ligand, f, is present per protomer. The behavior of such a system

can be described in terms of two independent functions: a state function $\langle r \rangle$ and a binding function $\langle y \rangle$ corresponding, respectively, to the fraction of protomers in the R state and to the fraction of sites to which the ligand is actually bound. These functions can be derived by the following two methods.

Method A.—Let $\langle y \rangle$ be the fraction of protomer sites occupied by the ligand f. The ligand binding energy is J_S or J_R, according to the state of the protomer. Let ϵ be the energy required to promote one protomer from S to R when all other protomers are in state S.

We make the molecular field approximation:[10] If the fraction $\langle r \rangle$ of the protomers are in state R, then the promotion energy is $\epsilon - \eta\langle r \rangle$, where $\eta\langle r \rangle$ is the mean interaction with all other R protomers.

In this model the grand partition function per protomer is[11]

$$\mathcal{Z} = (1 + \lambda e^{\beta J_S}) + (1 + \lambda e^{\beta J_R}) e^{-\beta(\epsilon - \eta\langle r \rangle)}, \tag{1}$$

where $\lambda \equiv e^{\beta\mu}$ = absolute activity of the ligand; $\beta \equiv 1/K_B T$. For an ideal system λ is directly proportional to the concentration of the ligand.

From (1) we obtain $\langle y \rangle$ and $\langle r \rangle$ by $\langle y \rangle = \lambda \partial(\log \mathcal{Z})/\partial\lambda$ and

$$\langle r \rangle = \mathcal{Z}^{-1} [(1 + \lambda e^{\beta J_R}) e^{-\beta(\epsilon - \eta\langle r \rangle)}], \tag{2}$$

a transcendental equation for $\langle r \rangle$. For most of the calculation we selected a set of values η, J_R, J_S, β, and then solved numerically for $\langle r \rangle$ and $\langle y \rangle$ as a function of λ.

Method B.—Consider a single protomer in a system of interacting protomers. The free energy ΔF of the transition $(S \rightleftharpoons R)$ depends on the fraction of protomers which are already in the R state. It may be shown[12] that

$$\Delta F = (\epsilon - \eta\langle r \rangle). \tag{3}$$

The isomerization constant $l' = \langle s \rangle / \langle r \rangle$ is then simply

$$l' = \exp[\beta(\epsilon - \eta\langle r \rangle)] = l\Lambda^{\langle r \rangle}, \tag{4}$$

where $l = e^{\beta\epsilon}$ and $\Lambda = e^{-\beta\eta} = e^{-N_0\eta/RT}$.

Binding of a ligand f to a single protomer is expressed by the following equilibria

$$R_1 \rightleftharpoons R_0 + f \qquad \begin{matrix} R_0 \rightleftharpoons S_0 \\ \text{and} \end{matrix} \qquad S_1 \rightleftharpoons S_0 + f,$$

and equations $\langle s_0 \rangle / \langle r_0 \rangle = l'$,

$$k_R = [f]\langle r_0 \rangle / \langle r_1 \rangle, \qquad \text{and} \qquad k_S = [f]\langle s_0 \rangle / \langle s_1 \rangle,$$

where k_R and k_S are the microscopic dissociation constants of R_1 and S_1, respectively.

The binding function of f is then simply:

$$\langle y \rangle = \frac{\langle r_1 \rangle + \langle s_1 \rangle}{\langle r_0 \rangle + \langle r_1 \rangle + \langle s_0 \rangle + \langle s_1 \rangle} = \frac{\alpha(1 + l\Lambda^{\langle r \rangle} c)}{1 + \alpha + l\Lambda^{\langle r \rangle}(1 + \alpha c)} \tag{5}$$

and the state function:

$$\langle r \rangle = \frac{\langle r_0 \rangle + \langle r_1 \rangle}{\langle r_0 \rangle + \langle r_1 \rangle + \langle s_0 \rangle + \langle s_1 \rangle} = \frac{1 + \alpha}{1 + \alpha + l\Lambda^{\langle r \rangle}(1 + \alpha c)} \tag{6}$$

with $\alpha = [f]/k_R$ and $c = k_R/k_S$.

Comparison and Generalization of Methods A and B.—With the following change of variable, $\alpha = \lambda e^{\beta J_R}$; $c = e^{\beta(J_S - J_R)}$; $l = e^{\beta\epsilon}$; and $\Lambda = e^{-\beta\eta}$, equations (1) and (2) become identical with equations (5) and (6). Methods A and B are equivalent; however, method B is expressed in terms of parameters more accessible to biological experimentation.[2]

Equations (5) and (6) can be generalized for the case in which two ligands f_1 and f_2 bind to the same protomer:

(1) f_1 and f_2 bind to the same receptor site (*mutual exclusion by steric hindrance*):

$$\langle r \rangle = \frac{1 + \alpha_1 + \alpha_2}{(1 + \alpha_1 + \alpha_2) + l\Lambda^{\langle r \rangle}(1 + \alpha_1 c_1 + \alpha_2 c_2)} \tag{7}$$

and

$$\langle y_1 \rangle = \frac{\alpha_1(1 + l\Lambda^{\langle r \rangle} c_1)}{(1 + \alpha_1 + \alpha_2) + l\Lambda^{\langle r \rangle}(1 + \alpha_1 c_1 + \alpha_2 c_2)}, \tag{8}$$

where the subscript 1 or 2 refers to the ligand f_1 or f_2, respectively.

(2) f_1 and f_2 bind at topographically distinct receptor sites (*allosteric interactions*):[13]

$$\langle r \rangle = \frac{(1 + \alpha_1)(1 + \alpha_2)}{(1 + \alpha_1)(1 + \alpha_2) + l\Lambda^{\langle r \rangle}(1 + \alpha_1 c_1)(1 + \alpha_2 c_2)} \tag{9}$$

and

$$\langle y_1 \rangle = \frac{\alpha_1(1 + \alpha_2) + \alpha_1 l\Lambda^{\langle r \rangle}(1 + \alpha_2 c_2)c_1}{(1 + \alpha_1)(1 + \alpha_2) + l\Lambda^{\langle r \rangle}(1 + \alpha_1 c_1)(1 + \alpha_2 c_2)}. \tag{10}$$

Our theory, based on the molecular-field approximation, is valid for one-, two-, or three-dimensional lattices. Alternative approximations have been developed for this general problem but the exact solution exists only for the one-dimensional model.[14]

In the following discussion, attention will be focused on the two-dimensional structures widely represented in living organisms by the biological membranes.

Discussion.—The proposed theory has been designed primarily to account for the *cooperative phenomena* accompanying the binding of ligands to a membrane: indeed, Figures 1 and 2 show that the predicted state and binding functions, $\langle r \rangle$ and $\langle y \rangle$, exhibit an S shape characteristic of cooperative interactions. In our simple formulation the cooperative character of the response is encompassed in a single parameter $\Lambda = e^{-\beta\eta}$. When $\Lambda = 1$, there is no lattice constraint, the protomers are independent, and both the $\langle r \rangle$ and $\langle y \rangle$ curves are hyperbolas. When Λ decreases, the $\langle r \rangle$ and $\langle y \rangle$ curves exhibit more and more cooperativity. For a critical value of Λ both curves become discontinuous (Fig. 2). This critical value $\Lambda_c = e^{-4} = 0.01831$ corresponds to a critical value $N_0\eta_c = 4RT$ and is independent of l and c. Such a discontinuity, which is also found with other two-dimensional models (Ising model), can be interpreted as a *phase transition* similar to the one which occurs between a gas and a liquid [$\langle r \rangle$ (resp. α) plays the role of the density (resp. pressure)]. In the present case, and at the critical concentration α_c of the ligand, the transition occurs

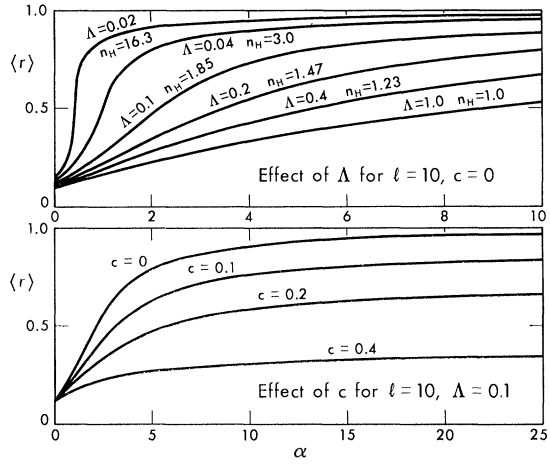

FIG. 1.—Effects of Λ and c on the shape and limits of the "state" function representative curve; n_H is the maximum value of the Hill coefficient defined as the derivative d log $[(\langle r\rangle_{max} - \langle r\rangle)/(\langle r\rangle - \langle r\rangle_{min})]/d$ log α.

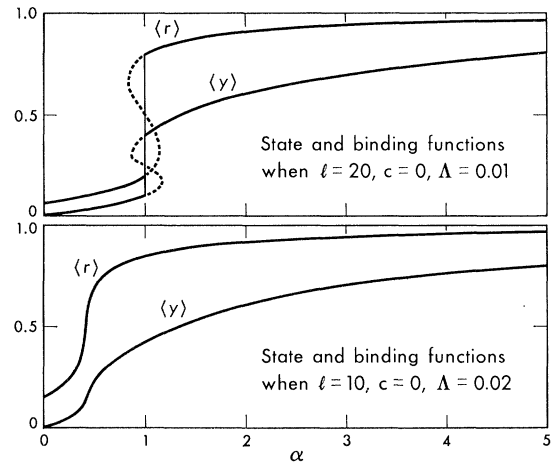

FIG. 2.—"State" and "binding" functions [eqs. (5) and (6)] in the case of a "graded" response (*lower figure*) and of an "all-or-none" response (*upper figure*). In the upper figure, the two stable states of the membrane are represented by the solid $\langle r\rangle$ curves. Their extrapolations as metastable states lie on the broken lines (the vertical solid line, defining a critical concentration, α_c, is drawn according to the general theory of phase transitions[10, 14]).

between two stable states of the membrane corresponding to the postulated two conformational states of the protomer.

If only nearest-neighbor protomers interact, then the molecular field theory leads to the following interpretation of the parameters:

$$l = \exp[z\beta(\epsilon_{RS} - \epsilon_{SS})] \quad \text{and} \quad \Lambda = \exp[z\beta(\epsilon_{RR} - 2\epsilon_{RS} + \epsilon_{SS})],$$

where z is the *number* of nearest neighbors, ϵ_{RR} (resp. ϵ_{RS} or ϵ_{SS}) is the energy of interaction between protomers in the conformations R and R (resp. R and S or S and S). The parameter l becomes the isomerization constant of a protomer in an environment of S protomers, and the parameter Λ characterizes the constraint imposed by the nearest neighbors. The critical value $N_0\eta_c = 4RT$ leads to a critical value for the free energy of interaction between protomers. It is of interest that the value found at 25°C, $N_0\eta_c = 2.36$ kcal/mole, is of the same order of magnitude as the variation of free energy of interaction between subunits observed in the allosteric transition of an oligomer [about 3.0 kcal for the sheep hemoglobin].[11]

The model thus predicts the two classes of responses exhibited by biological

membranes: a "graded" response or an "all-or-none" response depending on Λ and thus on the free energy of interaction between protomers.

Other predictions of our model may be of interest: For example, as shown in Figure 1, the asymptotic limit of the $\langle r \rangle$ function, for saturating levels of ligand, is sensitive to the value of c, the coefficient of nonexclusive binding.[16] This prediction is of relevance when one considers the response of excitable membranes to different pharmacologic agents[17] and, in particular, the observation that various related drugs provoke different maximal responses of the membrane.[18, 19]

Equations (7)–(10) give a simple mathematical description of the classical synergistic or antagonistic (competitive or noncompetitive) interactions between drugs, as observed with various categories of excitable membranes.[18]

At this point, we would like to emphasize some basic assumptions of our theory. (1) We assume that conformational changes of the protomer not only pre-exist the ligand binding, but are not fundamentally different whether the ligand is bound or not. Our model is thus essentially different from other models which postulate that the conformational alterations of macromolecular receptors are "induced," i.e., are consecutive to the binding of ligands.[20] As a consequence and as shown in Figure 2, curves $\langle r \rangle$ and $\langle y \rangle$ versus α should be, in many instances, distinct from each other.[21] The experimental test of this specific prediction should be relatively simple.[22] (2) Our model also differs from the model of Monod, Wyman, and Changeux[2] for allosteric oligomers, since we postulate that in a membrane different conformational states of the protomer may coexist close to each other. However, with these authors, we relate the cooperativity of a structure to the very arrangement of its constitutive protomers, in our case, the organization into a lattice possessing properties of translational symmetry.

Finally, we would like to suggest that a number of important biological phenomena seem to be related to the highly cooperative structure of cell membranes: For instance, the initiation and propagation of nerve impulses, the specific "killing" of some bacterial cells by a single molecule of colicine,[23] and several all-or-none processes[24] related to phage infection, bacterial conjugation, and fertilization.

Summary.—The cooperativity of biological membranes is discussed in terms of their lattice structure. A simple formulation is proposed for the relationships between the conformational transitions of the repeating units and the binding of ligands.[25]

* The work of Dr. Changeux was supported by U.S. Public Health Service research grant GM 12159 from the National Institutes of General Medical Sciences and by a National Science Foundation research grant GB 4810X; the work of Miss Tung and Dr. Kittel was supported by National Science Foundation grant GP-6345 and Office of Naval Research contract Nonr-3656(30).

† On leave from the Pasteur Institute, Paris, France. Dernham Fellow of the American Cancer Society, California Division.

‡ On leave from Département de Biologie, Centre d'Etudes Nucléaires de Saclay, B.P. No. 2, 91 Gif-sur-Yvette, France.

[1] Katz, B., in *Nerve, Muscle and Synapse* (New York: McGraw-Hill, 1966).

[2] Monod, J., J. Wyman, and J. P. Changeux, *J. Mol. Biol.*, **12**, 88 (1965).

[3] Green, D. E., and J. F. Perdue, these PROCEEDINGS, **55**, 1295 (1966); Korn, E., *Science*, **153**, 1491 (1966).

[4] Singer, S. J., personal communication.

[5] Kavanau, J., in *Structure and Function in Biological Membranes* (New York: Holden-Day, Inc., 1965); and Kavanau, J., in *Recent Progress in Surface Science*, ed. J. F. Danielli, K. G.

Pankhurst, and A. C. Riddiford (New York: Academic Press, 1964), vol. 1. See also Del Castillo, J., A. Rodriguez, C. A. Romero, and V. Sanchez, *Science*, **153**, 185 (1966); Delbrück, M., and W. Reichardt, in *Cellular Mechanisms in Differentiation and Growth*, ed. D. Rudwick (Princeton University Press, 1956); Lehninger, A. L., *Physiol. Rev.*, **42**, 467 (1962).

[6] Higman, H. B., T. R. Podleski, and E. Bartels, *Biochim. Biophys. Acta*, **75**, 187 (1963).

[7] The extreme stereospecificity of recognition of certain membrane receptors [see Amoore, J. E., "Current status of the steric theory of odor," *Ann. N.Y. Acad. Sci.*, **116**, 457 (1964)] can usefully be compared with the specificity of the regulatory sites on allosteric proteins [see Monod, J., J. P. Changeux, and F. Jacob, *J. Mol. Biol.*, **6**, 306 (1963)].

[8] Several of these statements are similar to those proposed by Monod, Wyman, and Changeux[2] and by Tasaki, I., I. Singer, and T. Takenata, *J. Gen. Physiol.*, **48**, 1095 (1965).

[9] The validity of this assumption has been extensively discussed by Lumry, R., R. Biltonen, and J. Brandts, *Biopolymers*, **4**, 917 (1966).

[10] Strässler, S., and C. Kittel, *Phys. Rev.*, **139**, A758 (1965); Hill, T. L., *Statistical Mechanics* (New York: McGraw-Hill, 1956), pp. 318–327.

[11] Throughout this paper we use a standard notation: K_B (Boltzmann constant); N_0 (Avogadro number); $R = N_0K_B$ (gas constant); T (absolute temperature); $\beta = 1/K_BT$.

[12] The identification of ΔF with $\epsilon - \eta\langle r\rangle$ in (1) is rigorous on this model. The partition function of the entire membrane may be divided as $\mathcal{Z} = \mathcal{Z}_1 + \mathcal{Z}_2$, where $\mathcal{Z}_1$ includes all terms with a particular site in state S, and $\mathcal{Z}_2$ has all terms with that site in R. Then $\mathcal{Z} = e^{-\beta F_1} + e^{-\beta F_2} \equiv e^{-\beta F_1}(1 + e^{-\beta\Delta F})$; it is exactly $1 + e^{-\beta\Delta F}$ that we write as $1 + e^{-\beta(\epsilon - \eta\langle r\rangle)}$ in Method A.

[13] With $\Lambda = 1$ these equations are formally identical to the equations proposed by Monod, Wyman, and Changeux[2] for allosteric oligomers.

[14] An extensive mathematical discussion of this problem in a different context can be found in Hill, T. L., *J. Chem. Phys.*, **20**, 1259 (1952) and in Hill, T. L., *Introduction to Statistical Thermodynamics* (New York: Addison-Wesley, 1960).

[15] Wyman, J., in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 28 (1963), p. 483.

[16] Rubin, M., and J. P. Changeux, *J. Mol. Biol.*, **21**, 265 (1966).

[17] Changeux, J. P., *J. Mol. Pharmacol.*, **2**, 369 (1966).

[18] Ariëns, E. J., and A. M. Simonis, *J. Pharm. Pharmacol.*, **16**, 137 (1964).

[19] The introduction of an "intrinsic activity" of the drug [(Ariëns, E. J., *Arch. Intern. Pharmacodyn.*, **99**, 32 (1954)] is no longer necessary.

[20] Pauling, L., these PROCEEDINGS, **21**, 186 (1935); Belleau, B., *J. Med. Chem.*, **7**, 776 (1964); Koshland, D. E., in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 28 (1963), p. 473; Mackay, D., *J. Pharm. Pharmacol.*, **18**, 201 (1966).

[21] Such a method has already been used for the demonstration of the highly concerted character of the allosteric transitions of an oligomeric protein (Changeux, Gerhart, Rubin, and Schachman, manuscript in preparation).

[22] We have purposely not specified in this discussion the nature of the ligands or the type of bonds involved in the conformational transitions of the protomers. Ligands may be group specific (small ions, protons...) or stereospecific (synaptic transmitters...)..., or even macro-molecules (cellular proteins, hormones, colicins,...). The corresponding specific sites may be located on the internal or on the external surface of the protomer. Conformational transitions of a protomer are understood as any reversible reorganization of its three-dimensional structure which may affect, in addition to its affinity toward specific ligands and its relationship with the neighboring protomers, some other characteristic function (as, for example, its involvement into permeability processes). Our theory, stated in a general form, should be adapted to each particular category of specific membrane and the observed response expressed as an $\langle r\rangle$ or "state" function.

[23] Nomura, M., these PROCEEDINGS, **52**, 1514 (1964).

[24] Membrane "phase transition" may become *permanent* as a result of the insertion of protomers of a different unit size, the structural modification of some of the native protomers by gain or loss of constitutive subunits, etc. The number of protomers required to be altered in this process does not have to be high, and eventually the modification of a single one could be sufficient to bring a dramatic change of the membrane response. The permanent modification of a synapse after a single conditioning impulse might operate, in a first step, through such a mechanism related to

the "all-or-none" transprinting of neurons postulated by Szilard in his theory "on memory and recall." (Szilard, L., these PROCEEDINGS, **51,** 1092 (1964).)

[25] The authors are greatly indebted to Dr. M. Delbrück and Dr. G. Adam for valuable suggestions and comments.